



Please adhere to good laboratory practice while following this guide, e.g. to load RNA into the Ncapsulate QCX completely under laminar flow, wearing gloves.

Need support? Feel free to reach out to us at customer.service@quantoom.com

About this kit

This Ncapsulate[®] **combo** kit is a research use only kit that allows the encapsulation of 1 mg mRNA or saRNA with the QCX-002 proprietary delivery system from Quantoom Biosciences. The QCX delivery system is a lipid-like nanoparticles composed of a proprietary ionizable component (QCX-002) and three other lipids (a helper lipid, a sterol and a PEGylated lipid). The kit contains five components: lipid 1, lipid 2, lipid 3, lipid 4 and an encapsulation buffer.

Pre-requisites not supplied

- **Equipment:** Ignite Precision NanoSystems NanoAssemblr, Precision NanoSystems cartridges, dialysis cartridges, regular lab items, vortex, thermoshaker, PVDF filter 0.22 µm, Amicon tubes (optional)
- **Consumables:**
 - Filtered RNA solution (concentration of ≥ 800 µg/mL in the Citrate 1 mM pH 3.8)
- **Reagent:**
 - Absolute ethanol 99%
 - TRIS 20mM sucrose 8% w/v pH 7.4

Storage

- The entire kit will be shipped at -20°C.
- Lipid powders are stored at -20°C, and freeze-thaw cycles should be avoided as much as possible.

Process overview

Table 1: Visualization of components present in the QCX Formulation combo Kit (for 1mg of RNA formulated)

Lipid mix and RNA mix preparation		Microfluidic mixing		Dialysis	Amicon tubes concentration (optional)	Filtration
Lipid mix preparation RT	RNA mix preparation RT	Formulation mixing RT	Dialysis Overnight 4°C	Concentration 4°C	PVDF 0,22 µm RT	
Components	Lipid 1	Lipid 2	Lipid 3	Lipid 4	Encapsulation buffer	
Labels						
Storage	-20°C				4°C	
Quantity	16 mg	11 mg	10 mg	3 mg	10 mL	

Protocol for Producing RNA-LLPs

This Ncapsulate[®] **combo** kit is intended for research use only and not for in-human use. RNA encapsulated lipid-like nanoparticles (RNA-LLPs) are prepared by mixing an ethanolic solution of lipids with an aqueous solution of RNA in slightly acidic environment. Upon mixing, the change in polarity of the environment induces the aggregation of lipids into nanoparticles.

1. Lipid mix preparation

1. Set the thermomixer at 35°C.
2. Based on Table 2, solubilize each lipid in an exact volume of Ethanol in each lipid tube.

Table 2: Ethanol absolute volume for lipid solubilization preparation (µL)

Components	Lipid 1	Lipid 2	Lipid 3	Lipid 4
Volume of Ethanol (µL)	2000	1100	1000	300
Labels				

3. Incubate each lipid in the thermomixer at 35°C, sonicated or 400–800 rpm, 30 min.
4. Make sure the solution is completely clear before using it.

Lipid mix solution table

1. Choose the type of final RNA, injection method and quantity of RNA formulated.
2. Based on Table 3, add the exact volume of each lipid in an appropriate tube.

Table 3: Volume for lipid mix solution preparation (µL)

				Lipid 1 (µL)	Lipid 2 (µL)	Lipid 3 (µL)	Lipid 4 (µL)	Ethanol (µL)	Total (µL)
	RNA type	Injection method	Quantity (µg)						
Type of kit	mRNA	IV	1000	1701,4	303,4	572	148,2	225,1	2950
			500	937,2	167,1	315,1	81,6	124	1625
			100	273,9	48,8	92,1	23,9	36,2	475
		IM, in vitro	1000	816,7	911,2	806,4	161,7	254	2950
			500	449,8	501,9	444,2	89,1	139,9	1625
			100	131,5	146,7	129,8	26	40,9	475
	scRNA	IM	1000	1701,4	515,4	304,3	163,5	265,5	2950
			500	937,2	283,9	167,6	90,1	146,2	1625
			100	273,9	83	49	26,3	42,7	475
		IM, in vitro	1000	816,7	413,7	372,5	124,6	1222,5	2950
			500	449,8	227,9	205,2	68,6	673,4	1625
			100	131,5	66,6	60	20,1	196,8	475

2. RNA mix preparation & microfluidic mixing

1. Dilute the filtered RNA solution in encapsulation buffer to obtain a final RNA concentration of 133.33 µg/mL. Use the table below to determine the required final volume based on the target quantity of formulated RNA.

Table 4: RNA solution in Encapsulation Buffer preparation

Target quantity of formulated RNA (µg)	Required final volume of encapsulation buffer containing RNA (mL)	Target RNA concentration in final encapsulation buffer (µg/mL)
1000	10	133.33
500	5	133.33
100	1.5	133.33

2. For an optimal mixing, use the appropriate mixing equipment (microfluidic mixer, impingement jet mixer, etc...).
3. Follow the supplier's instructions regarding the "Total Flow Rate - TFR", "Flow Rate Ratio - FRR", "Pre-waste" and "Post-waste".
4. If you are using the Ignite Precision NanoSystems NanoAssemblr Ignite for microfluidics, please note these settings:

Table 5: Ignite Precision NanoSystems NanoAssemblr parameters

Mixer	Quantity (µg)	TFR (mL/min)	FRR	Volume to formulate (mL)	Pre-waste (mL)	Post-waste (mL)	Type of syringe RNA used (mL)	Type of syringe lipid used (mL)
NanoAssemblr	1000	12	3	11	0.95	0.05	10	3
NanoAssemblr	500	12	3	5.7	0.65	0.05	5	3
NanoAssemblr	100	12	3	1.6	0.45	0.10	3	1

5. Put the NanoAssemblr cartridge into the dedicated space.
6. Draw up the RNA solution with a syringe fitted with a needle.
7. Remove the needle and insert the syringe into the tip of the NanoAssemblr cartridge.

Warning:

- If in the parameters, FRR is encoded 3:1, insert the RNA syringe into the left tip (C) to respect the FRR ratio 3:1.

8. Draw the lipid mix preparation with a syringe fitted with a needle.
9. Remove the needle and insert the syringe into the tip of the NanoAssemblr cartridge.

Warning:

- If in the parameters, FRR is encoded 3:1, insert the lipid mix syringe into the right tip (R) to respect the FRR ratio 3:1.
10. Put two 15 mL falcons on the arm (one for the waste, and one for the sample).
 11. Once parameters are good, press Start and wait until the end of the formulation.
 12. Your formulation is now ready for the next steps.

3. Dialysis

1. Prepare a volume of TRIS 20 mM, sucrose 8% w/v, pH 7.4 equal to 250X the volume of formulation recovered after microfluidic mixing..
2. Put the TRIS 20 mM sucrose 8% w/v pH 7.4 in a beaker with a magnetic stir bar.
3. Prime dialysis cartridge with an adapted volume (cut-off 20k MWCO) for 5 min into the TRIS 20 mM sucrose 8% w/v pH 7.4.
4. After priming, load LLPs aseptically with a syringe fitted with a needle.
5. Dialyze overnight with a gentle mixing in a cold room (4°C).
6. The next day, remove LLPs from the dialysis cassette aseptically with a syringe fitted with a needle.
7. Put your sample in an appropriate tube.

4. Amicon tubes concentration (optional)

1. Aseptically, fill out the Amicon ultra-filtration 15 mL (30 kDa molecular weight cut-off) with 5 mL of TRIS 20mM sucrose 8% w/v pH 7.4.
2. Prime Amicon ultra-filtration 15 mL (30 kDa molecular weight cut-off) by centrifuging for 5min at 4000 X g at 4°C.
3. Discard the TRIS 20mM sucrose 8% w/v pH 7.4.
4. Fill out the Amicon ultra-filtration 15 mL (30 kDa molecular weight cut-off) with LLPs.
5. Centrifuge until the desired concentration is achieved.

5. Filtration

1. Filter LLPs using PVDF syringe filters (0.22 µm cut-off - 13 mm filter).
2. Put your sample in an appropriate tube.
3. LLPs are ready to be used.

Abbreviations

- **IV:** intra-venous
- **IM:** intra-muscular
- **RT:** room temperature
- **N/A:** not applicable
- **LLP:** lipid-like nanoparticles

